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Meshcheryakov, A. YA.

Opyt polucheniya vysokospetsifichnoi pretsipitiruyushchei sibiryaxvennoi syvorotki

[Experimental] obtaining of highly species-specific precipitative anthrax sera]

Vsesoyuznyi Institut Eksperimental'noi Veterinarii. Trudy 25:384-398. 1961.
41.9 R923

(In Russian)

Differentiation of Bac. anthracis from anthracoids and other related species of spore-forming soil aerobic microbes has, up to [illegible], continued to be complicated.

In diffrential diagnosis of Bac. anthracis from similar spore-forming aerobic microbes, a whole complexity of different investigative methods has been used. In this investigative complexity, great importance is attributed to precipitation reaction (RP) which proved useful in the discovery of species-specific products of the causal agent of anthrax in the decomposed organs and skin of animals that had died from this infection, as well as in different products of animal origin, while other methods of investigation produced negative results. The

Junior Scientific Worker, Laboratory of Microbiology (Laboratoriya Mikrobiologii)

high sensitivity and specificity of this reaction has been confirmed by experience acquired in its use in all countries of the world in the course of many tens of years. In addition, Ascoli back in 1910 noted that precipitative anthrax sera produce group reaction with extracts [obtained] from anthracoids and other closely related spore-forming soil microbes.

Reports on this type of observations have since then appeared many times in the press.

E. Valenti, in 1911, arrived even at the conclusion that precipitation reaction was useless in differentiation of Bac. anthracis from anthracoids. The following year, Schutz and Pfeiler reported that highly active, precipitative anthrax sera which they had prepared had reacted not only with antigens of Bac. anthracis, but also with antigens of pseudoanthrax microbes. A non-specific reaction with pseudoanthrax microbes (7 strains) was obtained also by Pfeiler and Dreischer. Their attempt to differentiate Bac. anthracis from pseudoanthrax microbes, by means of alternate adsorption and exhaustion of the anthrax serum by extracts [obtained] from pseudoanthrax and anthrax microbes, failed to produce positive results. [Begin p. 385]

A more detailed comparative serological study of the precipitation reaction and the complement fixation reaction [RSK] of a large number of strains of the causal agents of anthrax and anthracoids was made by N. A. Pokshishevskii. He was the first to establish the presence of a cross precipitation

reaction [occurring] between the extracts obtained from the anthrax bacillus and from pseudoanthrax microbes and their precipitative sera, and to determine the limits of this reaction.

According to data of the commission which in 1939 had tested precipitative anthrax sera prepared by biomanufacturers and [according] to the investigations conducted by N. M. Nikiforova, M. V. Reve and F. E. Smirnov, in our country standard precipitative sera are not sufficiently active and produce non-specific indications up to 20-35%.

Takagi, in 1954, in testing a precipitative anthrax serum by precipitation reaction with different microbe and fungus species, obtained group reaction with some of them, even with species that are distantly related to the anthrax microbe (S. enteritidis, Br. abortus, Microc. aureus, B. coli and with certain fungi).

In 1956, O. Seidel and R. Strassmann reported that 16 of the anthracoid strains which they had investigated had given a positive precipitation reaction with anthrax sera.

In making a biochemical study of the antigenic structures of the anthrax microbe, as well as of closely related soil, spore-forming microbes, there was discovered the presence of species-specific,as well as of common antigens in the works of I. Tomcsik and M. Szonyott, W. Schaeffer and G. Sandor, G. Ivanovics, I. Tomcsik and G. Ivanovics, G. Ivanovics and L. Erdos, N. F. Gamaleya and I. E. Minkevich, M. V. Reve, and others.

The insufficient specificity of the precipitation reaction and its group character, which was found during the investigation of anthrax, have been responsible for numerous works [intended] for the improvement of the method of obtaining precipitative anthrax sera (S. A. Alekseev, 1912; M. Radkevich, 1925; N. A. Pokshishevskii, 1910; S. K. Bezzubets, 1927; R. M. Rozenberg and D. S. Romanov, 1927; F. A. Terent'ev, 1936; N. M. Nikiforova, 1935, 1947; F. S. Smirnov, 1945; S. G. Kolesov, 1955, and others).

The quality of precipitative anthrax sera was considerably improved when biomanufacturing plants which develop the immunization method suggested by S. G. Kolesov and V. I. Grachev were introduced in production; this method calls for live Bac. anthracis cultures with reduced virulence, yet immunogenic, instead of virulent [cultures] killed with formalin as required by the method used heretofore.

In the instruction on the preparation of precipitative anthrax sera, as well as in all veterinary reference works on microbiology and epizootiology, it is indicated that precipitative anthrax sera [used] in controls need not give a precipitation reaction with extracts (antigens) [Begin p. 386] of Bac. anthracoides and Bac. pseudoanthracis, at least not for 15 minutes of observation.

It must, however, be noted that despite the technological improvement, the precipitative sera released at the present time

still produce a certain percentage of non-specific indications and group reaction with pseudoanthrax and other closely related spore-forming aerobic microbes.

Between 1956 and 1958, at the Laboratory of Microbiology of the All-Union Institute of Experimental Veterinary Medicine [VIEV], we, under the supervision of Academician S. N. Muromtsev, conducted experimental work with a group of spore-forming aerobic microbes: Bac. anthracis, Tsenkovskii's 1st vaccine, Bac. anthracoides, Bac. pseudoanthracis, Bac. cereus, Bac. mesentericus, Bac. subtilis, Bac. megaterium, and Bac. mycoides; in this work we encountered the phenomenon that precipitative sera, which had been prepared at bio-manufacturing plants and also by us, produce group precipitation with the antigens of the above mentioned microbe species. We made a detailed study of this phenomenon of group precipitation. In addition, we were interested in the antigenic properties of the microbes used in the experiment, and in the precipitative properties of anthrax sera - the limits of their species-specific and group indications. We tested antigens of the above named microbes, according to the precipitation reaction, simultaneously with homologous and anthrax sera. In our experiments, we investigated: 11 strains of Bac. anthracis, a strain of Tsenkovskii's 1st vaccine, 5 strains of Bac. anthracoides, 4 strains of Bac. pseudoanthracis, 3 strains of Bac. subtilis, 2 strains of Bac. cereus, 6 strains of Bac.

mesenthericus 1 strain of Bac. megaterium, 1 strain of Bac. mycoides, and from non-spore-forming [microbes] - B. coli. From these microbes there were prepared precipitinogens according to the generally accepted method. Precipitative sera were obtained by means of hyperimmunization of rabbits. Besides the sera obtained from rabbits, there were tested different series of precipitative anthrax sera at the Tobol'sk and Orlov bio-manufacturing plants. Precipitation reaction was brought about according to the generally accepted method. In producing cross reactions it became clear that precipitinogens of the microbes listed give a positive precipitation reaction not only with homologous, but also with heterogenous sera, including anthrax sera prepared at bio-manufacturing plants. Group reactions we observed primarily with dilutions of antigens from 1:200 to 1:400 - 1:800, and only a few strains produced them in higher dilutions. Group reactions began, as a rule, somewhat later (after 2 - 15 minutes), then species-specific ones, depending on the species and microbe strain the quality of the sera and the antigen dilution.

The precipitative sera prepared at the Tobol'sk bio-manufacturing plant proved somewhat less active, but more species-specific (table 1, 2, 3), as compared with the precipitative sera released by the Orlov bio-manufacturing plant. [Begin p. 387].

The antigen and the sera of Tsenkovskii's 1st vaccine possessed strict specificity.

The results of these investigations are cited in table 1.

It is obvious from table 1 that different strains (Bac. anthracoides, Bac. pseudoanthracis, Bac. mesentericus, Tsenkovskii's 1st vaccine, Bac. cereus, Bac. subtilis) produce different degrees of group precipitation reaction with anthrax sera in antigen dilutions of 1:400 to 1:800. Two anthracoid strains (86 and 96) gave precipitation reaction with anthrax sera in antigen dilutions of 1:2500 - 1:3000 within the same space of time as anthrax with anthrax antigens. These strains are avirulent, they haemolyze media with blood, produce diffuse growth in broth, and are motile. As regards cultural-biochemical properties and, particularly, colony morphology and characteristics of reproduction, [these strains] do not differ from anthrax microbes; [they] completely extract anthrax precipitative sera and react poorly with homologous sera of other strains.

In our experiments, group precipitation reaction was observed with dilutions of antigens that were 5-10 times smaller than their dilution in a species-specific reaction. Thus, with precipitative sera of Bac. anthracoides, Bac. pseudoanthracis, Bac. cereus and Bac. mesentericus the titer of which was 1:2000 - 1:3000, group reactions were observed, primarily, with dilutions of antigens up to 1:400. Group precipitation reaction with anthrax sera, the titer of which was 1:4000-5000, did not exceed antigen dilutions of 1:800. However, two strains of anthracoids (86 and 96) produced it [the reaction] in an antigen dilution

up to 1:2500-3000, i. e. more than half of the anthrax serum titer.

These datas concur well with the results of investigations conducted by N. A. Pokshishevskii who established the fact that anthrax sera give a precipitation reaction with 1:50 dilutions of extracts [derived] from anthrax microbes, and with dilutions of extracts of pseudoanthrax microbes - from 1:5 to 1:20. The sera of pseudoanthrax microbes precipitated homologous antigens in a dilution of 1:50, and extracts from anthrax microbes - 1:10. On the basis of his own investigations, N. A. Pokshishevskii arrived at the conclusion that it is possible to differentiate between Bac. anthracis and pseudoanthrax microbes by the titer of their antigens, since, in his experiments, the extracts of Bac. anthracis precipitated with anthrax sera in dilutions that were 3-10 times larger than the extracts of pseudoanthrax microbes.

It follows from the data cited that anthrax, pseudoanthrax and other closely related microbe species have a species-specific fundamental antigen and a group antigen the content of which is approximately 5-10 times less. Corresponding precipitative sera contain species-specific and group precipitins in the same ratios. Consequently, [Begin p. 389], the microbes indicated have a serological similarity which, in different species and strains, is expressed in different degrees in relation to the quantity of group antigens. Species-specific antigens (the fundamental ones) determine the qualitative serological difference between the microbe species of a given group.

Table 1

Investigation of the antigenic properties of spore-forming soil microbes based on precipitation reaction

In numerical order	Microbe antigens: Name and number of strains	Dilutions	Precipitative sera: Bac. anthracis 1:5000*	Tsenkovskii's 1st vaccine 1:4000	Bac. anthracoides 1:3000	Bac. pseudoanthracis 1:3000	Bac. mesentericus 1:1500	Bac. cereus 1:1500	Bac. subtilis 1:400**
1	Bac. anthracis 63, 63A (4, 65, 66, 67, 68, 69	1:100	5"	2'	2'	3'	2'	4'	5'
		1:200	8"	4'	5'	8'	5'	10'	10'
		1:400	15"	8'	12'	16'	14'	-	+++
		1:800	20"	14'	-	-	-	-	-
		1:3000	1'	-	-	-	-	-	-
2	Tsenkovskii's 1st vaccine no. 18	1:100	3'	5"	-	-	-	-	-
		1:200	5'	6"	-	-	-	-	-
		1:400	12'	10"	-	-	-	-	-
		1:800	-	15"	-	-	-	-	-
3	Bac. anthracoides no. 86, 96	1:100	10"	-	35"	3'	2'	8'	10'
		1:200	13"	-	50"	6'	4'	12'	-
		1:400	20"	-	3'	14'	9'	-	-
		1:800	32"	-	10'	-	16'	-	-
		1:2500	3'	-	-	-	-	-	-
4	Bac. anthracoides no. 103, 103k	1:100	2'	-	2"	-	-	10'	-
		1:200	5'	-	3"	-	-	15'	-
		1:400	12'	-	5"	-	-	-	-
		1:800	-	-	12"	-	-	-	-
		1:2500	-	-	3'	-	-	-	-
5	Bac. anthracoides no. 8, 67	1:100	3'	-	40"	-	-	10'	-
		1:200	7'	-	2'	-	-	15'	-
		1:400	15'	-	5'	-	-	-	-
		1:800	-	-	12'	-	-	-	-

Table 1 continued

In numerical order	Microbe antigens: Name and number of strains	Microbe antigens: Dilutions	Precipitative sera: Bac. anthracis 1:5000*	Jasenkovskii's 1st vaccine 1:4000	Bac. anthracoides 1:3000	Bac. pseudo-anthracis 1:3000	Bac. mesentericus 1:1500	Bac. cereus 1:1500	Bac. subtilis 1:400**
6	Bac. pseudo-anthracis no. 16, 104k 104	1:100	3'	-	4'	2"	-	9'	-
		1:200	5'	-	10'	5"	-	15'	-
		1:400	12'	-	-	12"	-	-	-
		1:800	-	-	-	30"	-	-	-
7	Bac. pseudo-anthracis no. 98	1:100	4'	-	-	3'	-	-	-
		1:200	6'	-	-	5'	-	-	-
		1:400	14'	-	-	10'	-	-	-
		1:800	-	-	-	-	-	-	-
8	Bac. mesentericus no. 64, 66,66,70 72; 76	1:100	3'	-	-	-	4"	6'	-
		1:200	5'	-	-	-	6"	10'	-
		1:400	8'	-	-	-	14"	15'	-
		1:800	15'	-	-	-	32"	-	-
9	Bac. cereus (2 strains)	1:100	3'	-	10'	10'	-	4"	8'
		1:200	7'	-	-	-	-	5"	-
		1:400	15'	-	-	-	-	12"	-
		1:800	-	-	-	-	-	30"	-
10	Bac. subtilis no. 85, 85k, 720	1:100	3'	-	8'	12'	-	9'	1'
		1:200	6'	-	15'	-	-	15'	4'
		1:400	14'	-	-	-	-	-	12'
		1:800	-	-	-	-	-	-	-
11	Bac. mesaterium no. 89	1:100	6'	-	-	-	-	-	-
		1:200	10'	-	-	-	-	-	-
		1:400	-	-	-	-	-	-	-
		1:800	-	-	-	-	-	-	-

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Table 1 continued

In numerical order	Microbe antigens		Precipitative sera					
	Name and number of strains	Dilutions	Bac. anthracis 1:5000*	Tsenkovskii's 1st vaccine 1:4000	Bac. anthracoides 1:3000	Bac. pseudo-anthracis 1:3000	Bac. cereus 1:1500	Bac. subtilis 1:400**
12	B. [illegible] mycoides no. 183	1:100 1:200 1:400 1:800	10'	-	-	-	-	-
13	B. coli no. 31	1:100 1:200	-	-	-	-	-	-
	Control antigens:							
	1. Physiological solution	-	-	-	-	-	-	-
	2. Extract of [culture] medium	-	-	-	-	-	-	-

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Notes. 1. Time of the appearance of a positive reaction is indicated in minutes (') and in seconds (").

2. Control with normal horse and rabbit sera is negative.

3. Lines (-) in this and in the rest of the tables denote that tests were not made.

* Anthrax serum of the Orlov bio-manufacturing plant, series 89, 1955 and series 28, 1956.

** Titer of sera.

*** Reaction absent for 15 minutes. [Begin p. 390].

Conventional industrial precipitative sera are not strictly species-specific.

In our opinion, one of the causes of insufficient species-specificity of conventional anthrax precipitative sera is the heterogenous antigenic structure of anthrax microbes used in hyperimmunization: the content of group [antigens], as well as species-specific antigens, contained within them [the sera].

It follows from the above statements that, with the aid of conventional precipitative anthrax sera, the serological differentiation of Bac. anthracis from closely related species of spore-forming aerobic microbes, according to the antigenic titer and the time when reaction sets in, is connected with possible errors.

In connection with the wide-spread distribution in nature of pseudoanthrax and other, similar microbes, it can be hypothesized that in practice, particularly, in testing raw hides according to Ascoli's reaction, the group character of precipitation reaction could be one of the reasons of erroneous conclusions drawn with respect to anthrax.

There are many reports on this problem in the literature. On the other hand, as a result of the practice of certain diagnostic laboratories, it is known that imported raw hides, in particular those from Asian countries, produce a considerable percentage of non-specific reactions with anthrax sera when

tested for anthrax. In these cases, saprophytic spore-forming aerobic microbes are often isolated by means of bacteriological investigations.

Out of five sections of freshly dried [presnosukhaya] goatskins which we received from the Moscow Municipal Veterinary Bacteriological Laboratory, 4 specimens produced a distinctly positive precipitation reaction with anthrax sera. By means of bacteriological investigations, it was established that they had been vigorously seeded by Bac. anthracoides, Bac. pseudoanthracis and, to a lesser degree, by saprophytes of other species. In large dilutions, the extracts from these microbes produced a positive precipitation reaction with anthrax precipitative sera.

Attempts to raise the species-specificity of anthrax precipitative sera and of the precipitation reaction have been made long ago in testing for anthrax. N. A. Pokshishevskii and other researchers tried to increase the species-specificity of precipitative anthrax sera by means of diluting them with a physiological solution. N. M. Nikiforova used a 2% sodium chloride solution for the same purpose. The distrubance of the physico-chemical properties of sera is a shortcoming of this method.

B. S. Sukhoretskii, in an effort to remove this shortcoming from N. A. Pokshishevskii's and N. M. Nikiforova's

method, suggested diluting sera not with a physiological solution, but rather with the normal sera of rabbits, horses or cattle. This method, however, also failed to gain widespread use. In the first place, it is correlated with the need to have constantly [Begin p. 391] preliminarily tested normal sera and to use these sera to dilute precipitative sera and to determine their titer. In the second place, and this is most important, the fundamental cause of nonspecific (group) reactions, which is determined by the presence of group precipitins and group precipitinogens, cannot be removed by the method of sera dilution.

Proceeding from the characteristics of precipitation reaction, it must be emphasized that, in principle, the method of dilution of agglutinative sera is inapplicable with respect to precipitative sera.

Taking into account experimental preparation and widespread practical use of monospecific, monoreceptor and other strictly species-specific adsorbed agglutinative sera with respect to bacteria of the enterotyphus-paratyphoid group, we set ourselves the task of preparing a strictly species-specific, so-called monospecific precipitative anthrax serum by means of adsorption of biomanufacturing plant precipitative sera. We used in our work the principle of agglutinin adsorption according to Castellani [Kastelyani], and modified by us so as to be

applicable to precipitative sera. The essence of this method is contained in the proposition that a species-specific microbe which is added to an immune serum extracts not only its own principal agglutinins (precipitins), but also related ones (group agglutinins). A related microbe, however, is capable of binding only group agglutinins (precipitins), leaving almost all species-specific ones.

In our experiments, we used precipitative anthrax sera of the different series of the Orlov and Tobol'sk biomanufacturing plants (Tables 1, 2, 3) and the serum which we obtained from rabbits to be used against Bac. cereus (table 4). Anthrax sera were adsorbed by antigens of Bac. anthracoides and Bac. pseudoanthracis separately and in mixed form. The serum used against Bac. cereus was extracted by antigens of Bac. anthracoides and Bac. anthracis.

Three 24-hour agar cultures were used to prepare antigens. The skimmed microbe mass was autoclaved at 112-120° [C] for 30 minutes and then dried to a constant weight by one of the following methods: with the aid of lyophilization, in a vacuum exsiccator, or in a drying cabinet at a temperature of 40-50° [C]. The method of lyophilic drying is the more desirable one, because antigens prepared by this method are convenient to use, readily soluble and have a more standard nature. A microbe mass dried by other methods was preliminarily

ground into a fine powder. Sera adsorbed the powder-like or, better, preliminarily diluted 1:2-1:4 physiological solution of the microbe mass. In order to achieve complete adsorption of sera, there were tested different doses of antigens: 1:100; 1:200; 1:400; 1:1000; and 1:2000 ml sera. After adding an antigen, the sera were conserved with merthiolate [Begin p. 392], or with phenol and were kept for 3 hours at a temperature of 37° [C] and 20-25 hours at room temperature with periodic stirring up. Then the sera were centrifuged at 3-4 thousand rpm [revolutions per minute], or filtered through Seitz's filter. Further they were tested for completeness of adsorption and species-specificity according to the precipitation reaction with antigens of anthrax, pseudoanthrax and other closely related bacteria.

As a result of many experiments, we established that adsorption of precipitative anthrax sera by dry antigens (of the dry powder-like microbe mass) of Bac. anthracoides and Bac. pseudoanthracis, estimated at 250-400 mg of antigens per 100 ml of sera offers the possibility of obtaining a strictly species-specific (monospecific) serum deprived of group antibodies. [The name] monospecific sera implies precipitative sera from which heterogeneous antigens, with the aid of adsorption, have removed all group precipitins that were non-specific for bacteria of the given species. Such sera

react only with bacteria that posses a corresponding antigen.

In tables 2, 3, and 4 are presented summarized data of comparative testing of native monospecific precipitative sera and of those we obtained. From these tables it is obvious that monospecific sera produce precipitation reaction only with homologous antigens and do not contain group precipitins to all other representatives of this microbe group which we have investigated. The anthracoid strains 86 and 96 with which monospecific sera continued to react, but to a lesser degree than the original ones, are an exception.

The titer of monospecific sera depends on the activity and species-specificity of the original sera and usually is somewhat lower than in the latter.

In our experiments, a decrease in the sera titer by 1/5-1/6 of their original activity was observed after adsorption. The use of antigens for adsorption in higher doses (0.8-1 gm per 100 ml of sera) leads to an even more undersirable decrease in the sera titer.

Yet, smaller doses of antigens (0.05-0.1 gm per 100 ml of sera) do not insure complete extraction of group precipitins. By the use of the same method (by means of exhaustion on the part of dry antigens of Bac. anthracis and Bac. anthracoides in a dose of 150-200 mg per 100 ml of sera), we obtained a monospecific precipitative serum to be used against Bac. cereus.

Monospecific sera obtained in parallel tests with conventional native sera have always displayed strict species-specificity when microbe cultures used in the experiment were investigated. [Begin p. 393].

Table 2

Comparative testing of native and adsorbed monospecific anthrax precipitative sera according to precipitation reaction

In numerical order	Microbe antigens		Time of appearance of reaction with		Control sera
	Name	Dilution	Native sera*	Adsorbed sera 0.250:100 ml	Normal sera of a horse and a rabbit
1	Bac. anthracis ser. 52, 1955 Tobol'sk Bioplant	1:4000	8"	10"	-
2	Bac. anthracis no. 63,64,68	1:100	5"	8"	-
		1:200	8"	12"	-
		1:400	10"	20"	-
3	Bac. anthracoides, no.86, 96	1:100	10"	30"	-
		1:200	15"	1'	-
		1:400	25"	3'	
4	Bac. anthracoides, no. 8, 67, 103, 103k	1:100	2'	-**	
		1:200	5'	-	
		1:400	12'	-	-
5	Bac. pseudoanthracis, no. 16, 98, 104, 104k	1:100	2'	-	-
		1:200	5'	-	-
		1:400	14'	-	-
6	Bac. mesentericus, no. 64, 65 66, 70, 72, 76	1:100	2'	-	-
		1:200	5'	-	-
		1:400	10'	-	-
7	Bac. subtilis no. 85, 85k, 720	1:100	5'	-	-
		1:200	7'	-	-
		1:400	14'	-	-
8	Bac. cereus (2 strains)	1:100	3'	-	-
		1:200	8'	-	-
		1:400	15'	-	-
	Control antigens				
1	Physiological solution		-	-	-
2	Extract of [culture] medium		-	-	-

Note. Time of the appearance of a positive reaction is denoted in minutes (') and in seconds (")

* Sera of the Orlovo Bio-Manufacturing Plant, series, 88 and 89, 1955; series 9 and series 28, 1956.

Their titer prior to adsorption 1:4000 - 1:5000, after adsorption 1:3300 - 1:4200.

** Reaction absent for 15 minutes.

Table 3

Comparative testing of native and adsorbed monospecific anthrax precipitative serum, according to precipitation reaction

In numerical order	Microbe antigens		Time of appearance of reaction with		Control sera
	Name	Dilution	Native sera	Adsorbed sera 0.250:100 ml	Normal sera of a horse and a rabbit
1	Bac. anthracis ser.. 52, 1955 Tobol'sk bioplant	1:4000	14"	20"	-
2	Bac. anthracis no. 63, 64,68	1:100 1:200 1:400	10" 15" 20"	18" 25" 32"	- - -
3	Bac. anthracoides no. 86 96	1:100 1:200 1:400	30" 1,5' 3'	3' 6' -	- - -
4	Bac. anthracoides no. 8 67, 103, 103k	1:100 1:200 1:400	4' 7' 16'	-** - -	- - -
5	Bac. pseudoanthracis, no.16 98, 104, 104k	1:100 1:200 1:400	7' 12' -	- - -	- - -
6	Bac. mesentericus, no. 65, 66 70, 72, 76	1:100 1:200 1:400	3' 7' 15'	- - -	- - -
7	Bac. subtilis no, 85, 85k, 720	1:100 1:200 1:400	9' 15' -	- - -	- - -
8	Bac. cereus (2 strains)	1:100 1:200 1:400	7' 12' -	- - -	- - -
	Control antigens				
1	Physiological solution		-	-	-
2	Extract of [culture] medium		-	-	-

Note. Time of the appearance of a positive reaction is denoted in minutes (') and in seconds (").

* Sera of the Tobol'sk Bio-Manufacturing Plant, series 15 and 20, 1955. Titer prior to adsorption 1:4000, after adsorption 1:3500.

** Reaction absent for 15 minutes.

Table 4

Comparative testing of the native and adsorbed (monospecific) precipitative sera of Bac. cereus according to precipitation reaction

In numerical order	Microbe antigens		Time of appearance of reaction with		Control sera
	Name	Dilution	Native sera	Adsorbed sera 0.200:100 ml	Normal serum of a rabbit
1	Bac. anthracis, Ser. 52, 1955 Orlov Bio-Plant	1:4000	-	-	-
2	Bac. anthracis (7 strains)	1:100 1:200 1:400	6' 10' -	-** - -	- - -
3	Bac. anthracoides no. 86, 96	1:100 1:200 1:400	8' 12' -	- - -	- - -
4	Bac. anthracoides no. 67, 103, 103k	1:100 1:200 1:400	10' 15' -	- - -	- - -
5	Bac. pseudoanthracis, no. 16, 104	1:100 1:200 1:400	9' 15' -	- - -	- - -
6	Bac. subtilis no. 85, 720	1:100 1:200 1:400	12' 15' -	- - -	- - -
7	Bac. cereus (2 strains)	1:100 1:200 1:400	5" 9" 15"	7" 10" 20"	- - -
8	Bac. mesentericus no. 65, 70, 76	1:100 1:200 1:400	6' 10' 15'	- - -	- - -
	Control antigens				
1	Physiological solution		-	-	-
2	Extract of [culture] medium		-	-	-

Note. Time of appearance of positive reaction is denoted in minutes (') and in seconds(").

o Sera were obtained from rabbits. Titer prior to adsorption 1:1500, after adsorption 1:1200.

** Reaction is absent for 15 minutes. [Begin p. 396].

CONCLUSIONS

1. Bac. anthracis, Tsenkovskii's 1st vaccine, Bac. anthracoides, Bac. pseudoanthracis, Bac. mesentericus, Bac. cereus, Bac. subtilis and Bac. megaterium have, in addition to the the basic species-specific antigens, also somatic group antigens the content of which, depending on the microbe species and strain, is 5-10 times less, and their native precipitative sera contain group precipitins in similar correlations. They produce group precipitation reaction at the expense of these group antigens and precipitins.

2. The precipitative sera of Tsenkovskii's 1st vaccine possesses strict species-specificity and does not produce group precipitation reaction with saprophytic, spore-forming aerobic microbes.

3. The heterogeneous antigenic structure of anthrax microbes (coontent of species-specific and group antigens) which are used in hyperimmunization is one of the causes of deficient species-specificity of conventional precipitative anthrax sera produced on an industrial scale.

4. Anthrax sera produce group species-specific reactions primarily in antigen dilutions of 1:400-1:800, and only with distantly related strains of anthracoides in 1:2500-3000 [dilutions], but sera of pseudoanthrax microbes in antigen dilutions of 1:200-1:400 - dilutions 5-10 times less than the dilutions in species-specific reactions.

5. The insufficient species-specificity of an industrial precipitative anthrax serum does not permit conducting a strictly serological differentiation of Bac. anthracis from closely related spore-forming aerobic microbes.

6. By means of adsorption of conventional, native, precipitative sera by corresponding hetero-antigens (dry microbe mass), it is possible to obtain strictly species-specific (monospecific) precipitative sera.

7. By means of exhaustion of a bio-plant made precipitative anthrax serum by antigens of Bac. anthracoides and Bac. pseudoanthracis, in ratios of 250-400 mg of antigen per 100 ml of sera, there was obtained a monospecific anthrax serum. The titer of such a serum depends on the activity and species-specificity of the original serum, and, usually, is somewhat lower than [the titer] of the latter.

8. With the aid of an anthrax monospecific (adsorbed) serum, it is possible to conduct stricter serological indentification and differentiation of Bac. anthracis from spore-forming aerobic microbes.

9. We tested monospecific anthrax sera according to Ascoli's reaction for the purpose of determining whether or not materials were contaminated by the causal agent of anthrax.

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